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Mutagenic and Carcinogenic Potency of Diesel and Related Environmental Emissions: Salmonella Bioassay

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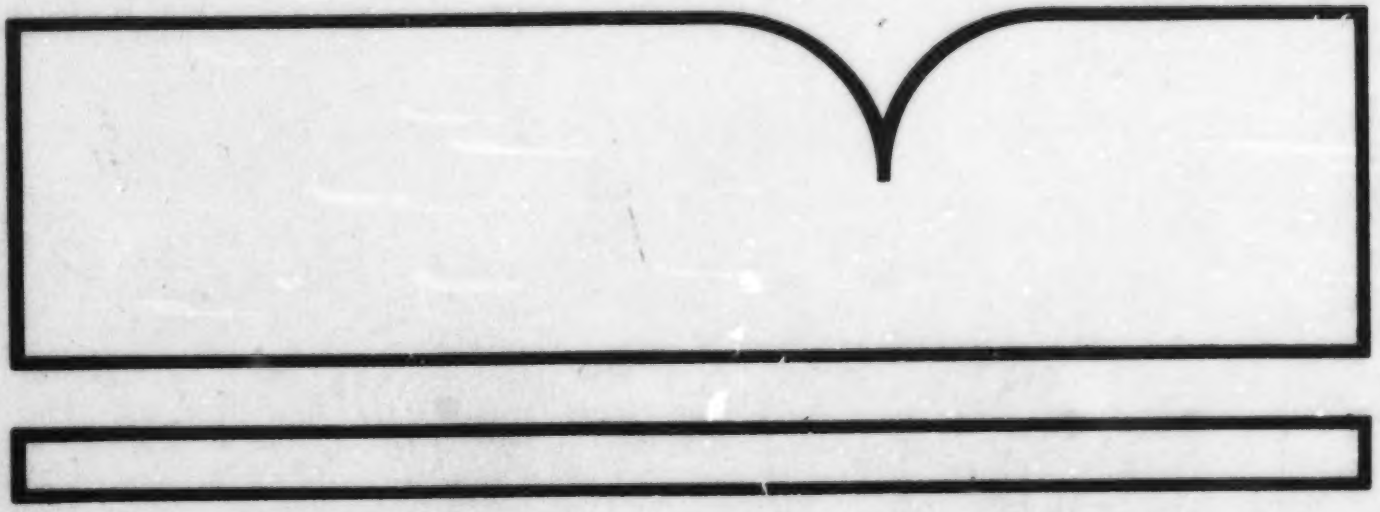

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16. ABSTRACT
Due to the expected increase in the percentage of diesel vehicles in the United States, the Environmental Protection Agency must evaluate the health effects associated with exposure to diesel emissions. Respirable particles from a variety of combustion sources have the potential of being carcinogenic and mutagenic. The objective of these studies was to determine the relative biological activity of the organic material adsorbed on these particles in vitro mutagenesis bioassays. The organic extracts from the following series of emission sources were bioassayed in the Salmonella assay for mutagenic activity: (1) a light-duty Oldsmobile diesel 350 engine; (2) a heavy-duty Caterpillar diesel engine; (3) a light-duty Nissan engine; (4) a Volkswagen Rabbit diesel; (5) cigarette smoke; (6) roofing tar; (7) coke oven; and (8) a gasoline catalyst Mustang. This paper provides a comparison of these sources within the Salmonella bioassay and also demonstrates how bacterial systems can be used as a quality assurance measure in vivo testing.

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JOURNAL ARTICLE

MUTAGENIC AND CARCINOGENIC POTENCY OF DIESEL AND RELATED ENVIRONMENTAL EMISSIONS: *SALMONELLA* BIOASSAY

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Due to the expected increase in the percentage of diesel vehicles in the United States, the Environmental Protection Agency must evaluate the health effects associated with exposure to diesel emissions. Respirable particles from a variety of combustion sources have the potential of being carcinogenic and mutagenic. The objective of these studies was to determine the relative biological activity of the organic material adsorbed on these particles in *in vitro* mutagenesis bioassays. The organic extracts from the following series of emission sources were bioassayed in the *Salmonella* assay for mutagenic activity: (1) a light-duty Oldsmobile diesel 350 engine; (2) a heavy-duty Caterpillar diesel engine; (3) a light-duty Nissan engine; (4) a Volkswagen Rabbit diesel engine; (5) cigarette smoke; (6) roofing tar; (7) coke oven; and (8) a gasoline catalyst Mustang. This paper provides a comparison of these sources within the *Salmonella* bioassay and also demonstrates how bacterial systems can be used as a quality assurance measure in *in vivo* testing.

Introduction

Bacterial systems have recently been used to demonstrate that organic mixtures from combustion processes, including diesel engines, are mutagenic (Huisingh *et al.*, 1979; Kier *et al.*, 1974). This study demonstrates a means of comparing the mutagenic potential of various sources with microbial test methods. Included within the problem of source to source comparison are variables within: (1) collection methods, (2) methods of chemical extraction, (3) proportion of mutagenic components within the mixture, (4) the amount of "masking" organics that are bactericidal, (5) the metabolizing ability of the indicator organism and exogenous activation systems, and (6) the sensitivity of the bioassay. Although these variables do exist, they can be controlled to some extent. In view of these considerations, this paper demonstrates the utility of comparing organics from various combustion sources. This study also demonstrates how bacterial systems can aid in the identification of active components and can be used as a quality assurance measure in *in vivo* testing.

Materials and Methods

The assay system used in this study was the *Salmonella typhimurium* plate incorporation test as developed by Ames *et al.* (1975). Four mutant strains of *Salmonella typhimurium* (TA98, T100, TA1535, and TA98-FR1) were used in this study. The nitroreductase deficient strain TA98-FR1 was obtained from Dr. Herbert Rosenkranz at the New York Medical College, Valhalla, NY, and the other strains were obtained from Dr. Bruce Ames at the University of California, Berkeley. All samples were tested in TA98, TA1535, and TA100. The diesel and cigarette smoke samples were assayed also with TA98-FR1.

The protocol described by Ames *et al.* (1975) was employed with minor modifications described below. All samples were tested with and without Aroclor-induced S-9 as described by Ames (3). Two minor modifications to the protocol were used. First, the minimal level of histidine was added to the plate media rather than to the overlay. Secondly, plates were incubated for 72 hours rather than 48 hours. Each plate was counted electronically using an Artek Model 880 automatic colony counter. The positive controls used for TA98 and TA98-FR1 were 2-nitrofluorene for without activation and 2-anthramine for the S-9 activation control. For TA100 and TA1535, sodium azide was substituted for the positive control without activation. All quality assurance procedures as described by Ames *et al.* (1975) and the Washington Committee on the

Discussion

Each organic sample from the various combustion source emissions was demonstrated to be mutagenic in the *Salmonella typhimurium* mutagenesis assay. This study demonstrates that emission organics can be compared within bacterial assay systems and that microbial assays can supply important supplemental information.

The bacterial assay demonstrated that the mutagenic substances associated with organic emissions were different for each type of emission. For example, the cigarette smoke condensate required activation before mutagenic activity was seen; however, most of the activity associated with the diesel samples was direct acting. Secondly, the range of activity found with the diesel sample spanned the range of activities associated with the comparative samples. Next, the lack of response in strain TA1535 suggests that most, if not all, of the activity is due to polynuclear frameshift mutagens and not to alkylating agents. The nitroreductase deficient strain demonstrated a difference between the two samples available for testing. The responses of TA98 and TA98-FR1 were markedly different with the diesel Nissan sample but quite similar with the cigarette smoke condensate. This would suggest that the particle bound organics from the Nissan diesel contains inactive nitro compounds that are reduced to an active form by nitroreductase enzymes which are found in TA98.

The calculation of relative potencies is a useful means of understanding the range of activity available from various combustion organics. As can be easily seen from Table 2, the maximum attainable difference within this study was seen between the Caterpillar and Nissan samples which demonstrated approximately 25-fold difference. The quality assurance portion of this study provides evidence that the active components in the microbial mutagenesis assay are stable when stored as the extract at $-70\,^{\circ}C$ for at least seven months.

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